REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
29 October 2014	**FINAL**	1 Aug 2011 - 31 July 2014

4. TITLE AND SUBTITLE
Coenzyme Q10: A New Treatment for Hemorrhagic Shock

5a. CONTRACT NUMBER
N/A

5b. GRANT NUMBER
HU0001-11-1-TS09

5c. PROGRAM ELEMENT NUMBER
N/A

6. AUTHOR(S)
Pierce, Janet D, CAPT USNR, NC, PhD, RN

5d. PROJECT NUMBER
N11-C02

5e. TASK NUMBER
N/A

5f. WORK UNIT NUMBER
N/A

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Kansas Medical Center
3901 Rainbow Blvd
Kansas City, KS 66160

8. PERFORMING ORGANIZATION REPORT NUMBER
N/A

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
TriService Nursing Research
Program, 4301 Jones Bridge RD
Bethesda, MD 20814

10. SPONSOR/MONITOR'S ACRONYM(S)
TSNRP

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
N11-C02

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES
N/A

14. ABSTRACT

Purpose: To examine the effects of ubiquinol (reduced form of Coenzyme Q10) in leukocytes, lungs, diaphragm, and microcirculation following hemorrhagic shock (HS) **Design:** Experimental. **Methods/Instrumentation:** Anesthetized rats were bled to induce HS by removing 40% of the blood volume over 60 minutes. The rats were resuscitated with blood and lactated Ringer's solution, with or without ubiquinol, and monitored for 120 minutes. Lungs and diaphragm were excised and harvested for hydrogen peroxide (H_2O_2) concentration and apoptosis analysis. Leukocytes were analyzed for mitochondrial superoxide (O_2) at baseline, end of shock, and 120 minutes following fluid resuscitation. In another set of experiments, leukocyte adherence and mast cell degranulation (MCD) was obtained. Vascular permeability was assessed and reactive oxygen species (ROS) in the venular walls were determined. **Sample:** Sprague Dawley male rats were used and randomly assigned to the control or experimental groups. There were 10 rats per group in each set of experiments. **Analysis:** Statistical significances were defined at a $p \leq 0.05$. Various types of nonparametric statistics were used. **Findings**: Mitochondrial leukocyte O_2 in the control group increased and there was a 30% rise at the end of the experiment, as compared to ubiquinol group. Similarly lung and diaphragm apoptosis in the control group was significantly higher. Diaphragmatic H2O2 in the control group was also significantly higher than the ubiquinol group. There were significant differences in leukocyte adherence, the MCD index, vascular permeability and microcirculation ROS production between the control and the ubiquinol groups. **Implications for Military Nursing:** Ubiquinol is a safe and easily administered supplement that prevents damage and reperfusion injury following HS. Attenuating damage to organs with the use of ubiquinol following HS could be used in military personnel.

15. SUBJECT TERMS hemorrhagic shock, ubiquinol, Coenzyme Q10, patient outcome					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON Debra Esty
a. REPORT UNCLASSIFIED	b. ABSTRACT UNCLASSIFIED	c. THIS PAGE UNCLASSIFIED	UU	104	19b. TELEPHONE NUMBER *(include area code)* 301-319-0596

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TriService Nursing Research Program Final Report Cover Page

Sponsoring Institution	TriService Nursing Research Program
Address of Sponsoring Institution	4301 Jones Bridge Road Bethesda MD 20814
USU Grant Number	HU0001-11-1-TS09
USU Project Number	N11-C02
Title of Research Study or Evidence-Based Practice (EBP) Project	Coenzyme Q10: A New Treatment for Hemorrhagic Shock
Period of Award	1 AUG 2011 - 31 JUL 2014
Applicant Organization	University of Kansas Medical Center
Address of Applicant Organization	3901 Rainbow Boulevard, Kansas City, KS 66160

Principal Investigator (PI) Military Contact Information

Duty Title

PI Civilian Work

Duty Title

Employer

Address

Telephone

Mobile Telephone

E-mail Address

PI Home Contact

Address

Telephone

Mobile Telephone

E-mail Address

Signatures

PI Signature  Date 29 OCT 2014

Mentor Signature Not Applicable (N/A) Date

Table of Contents

Abstract

Purpose: To examine the effects of ubiquinol (reduced form of Coenzyme Q10) in leukocytes, lungs, diaphragm, and microcirculation following hemorrhagic shock (HS).

Design: Experimental.

Methods: Anesthetized rats were bled to induce HS by removing 40% of the blood volume over 60 minutes. The rats were resuscitated with blood and lactated Ringer's solution, with or without ubiquinol, and monitored for 120 minutes. Lungs and diaphragm were excised and harvested for hydrogen peroxide (H_2O_2) concentration and apoptosis analysis. Leukocytes were analyzed for mitochondrial superoxide ($O_2^{\cdot-}$) at baseline, end of shock, and 120 minutes following fluid resuscitation. In another set of experiments, leukocyte adherence and mast cell degranulation (MCD) was obtained. Vascular permeability was assessed and reactive oxygen species (ROS) in the venular walls were determined.

Sample: Sprague Dawley male rats were used and randomly assigned to the control or experimental groups. There were 10 rats per group in each set of experiments.

Analysis: Statistical significances were defined at a $p \leq 0.05$. Various types of nonparametric statistics were used.

Findings: Mitochondrial leukocyte $O_2^{\cdot-}$ in the control group increased and there was a 30% rise at the end of the experiment, as compared to ubiquinol group. Similarly, lung and diaphragm apoptosis in the control group was significantly higher. Diaphragmatic H_2O_2 in the control group was also significantly higher than the ubiquinol group. There were significant differences in leukocyte adherence, the MCD index, vascular permeability and microcirculation ROS production between the control and the ubiquinol groups.

Implications for Military Nursing: Ubiquinol is a safe and easily administered supplement that prevents cellular damage and reperfusion injury following HS. Attenuating damage to organs with the use of ubiquinol following HS could be used in military personnel.

TSNRP Research Priorities that Study or Project Addresses

Primary Priority

Force Health Protection:	☐ Fit and ready force ☐ Deploy with and care for the warrior ☐ Care for all entrusted to our care
Nursing Competencies and Practice:	☒ Patient outcomes ☐ Quality and safety ☒ Translate research into practice/evidence-based practice ☐ Clinical excellence ☐ Knowledge management ☐ Education and training
Leadership, Ethics, and Mentoring:	☐ Health policy ☐ Recruitment and retention ☐ Preparing tomorrow's leaders ☐ Care of the caregiver
Other:	☐

Secondary Priority

Force Health Protection:	☐ Fit and ready force ☐ Deploy with and care for the warrior ☒ Care for all entrusted to our care
Nursing Competencies and Practice:	☐ Patient outcomes ☐ Quality and safety ☐ Translate research into practice/evidence-based practice ☐ Clinical excellence ☐ Knowledge management ☐ Education and training
Leadership, Ethics, and Mentoring:	☐ Health policy ☐ Recruitment and retention ☐ Preparing tomorrow's leaders ☐ Care of the caregiver
Other:	☐

Progress Towards Achievement of Specific Aims of the Study or Project

Findings related to each specific aim, research or study questions, and/or hypothesis:

We have completed data collection for all hypotheses related to AIM #1 and AIM #2. Below is the description of the completed achievement of our specific aims for this grant.

AIM #1: To examine the effect of CoQ10 in leukocytes, the lung, and the diaphragm as a treatment for hemorrhagic shock (HS).

Research Hypothesis 1a: Administering intravenous (IV) CoQ10 decreases leukocyte mitochondria reactive oxygen species (ROS) following HS.

In the last 12 months since our Year 2 annual progress report, we completed the measurements on superoxide production in leukocyte mitochondria to examine the effects of intravenous (IV) ubiquinol (reduced form of coenzyme Q10) in a hemorrhagic shock rat model. A synopsis of the method is as follows: monoclonal antibodies CD45 were used to identify leukocytes in the whole blood and MitoSox Red reagent to detect superoxide production in the mitochondria. As reported in the first annual report, we adjusted the protocol to defrost the MitoSox Red reagent at least 15 minutes before obtaining samples. This allowed the reagent to warm to room temperature. To preserve its reactivity, we prepared a new MitoSox Red reagent working solution from the stock sample right before we obtained samples at baseline, shock, and treatment periods. We gated 10,000 CD45 positive cells (leukocytes) and collected data on leukocyte mitochondrial superoxide by measuring mean fluorescent intensity (MFI) of MitoSox Red.[1,2]

As discussed in annual report Year 2, Figure 1 displays examples of flow cytometry results of leukocyte mitochondrial superoxide production at baseline, shock, and fluid resuscitation with or without ubiquinol. In this example, the MFI increased after hemorrhagic shock and increased following reperfusion of blood and Lactated Ringer's (LR). However, the

administration of IV ubiquinol before reperfusion of the blood and LR prevented the increase in superoxide production.

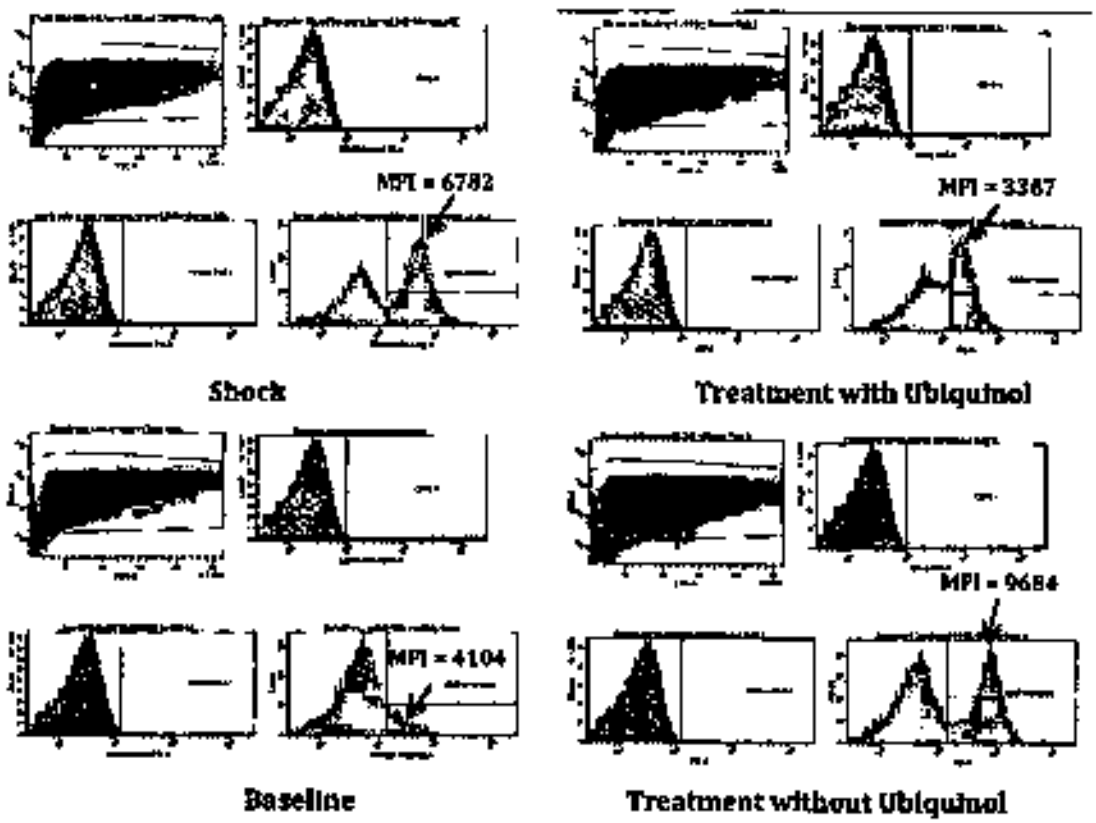


Figure 1. Examples of flow cytometry results of leukocyte mitochondrial superoxide production at baseline, shock, and fluid resuscitation with or without ubiquinol.

Table 1 displays MFIs of MitoSox Red at baseline, following hemorrhagic shock and fluid resuscitation with or without administering IV ubiquinol.

Table 1. Mean fluorescent intensity of MitoSox red at baseline, shock, and without or with ubiquinol treatment.

	Control	Ubiquinol
Baseline	5653.5 ± 306.2	5617.5 ± 242.5
Shock	6593.0 ± 377.8	6491.1 ± 265.8

Treatment	7227.9 ± 534.5	4687.2 ± 265.4

Data are expressed as mean ± standard error of mean, n = 10.

As shown in Table 1, leukocyte mitochondrial superoxide levels in both groups (control vs. ubiquinol) at baseline were comparable (5653.5 ± 306.2 vs. 5617.5 ± 242.3, respectively). Following 60-minute hemorrhagic shock, leukocyte mitochondrial superoxide production increased significantly from baseline in both the control (6593.0 ± 377.8) and ubiquinol (6491.1 ± 265.8) groups. Without administering IV ubiquinol during fluid resuscitation in the control group, superoxide production continued to increase and there was 30% of elevation at the end of experiment, as compared to baseline. In contrast, superoxide levels reduced in the ubiquinol group after 120-minute fluid resuscitation period. The level was even lower than the baseline level. The difference in superoxide production at the end of fluid resuscitation between the two groups was statistically significant ($p < 0.001$).

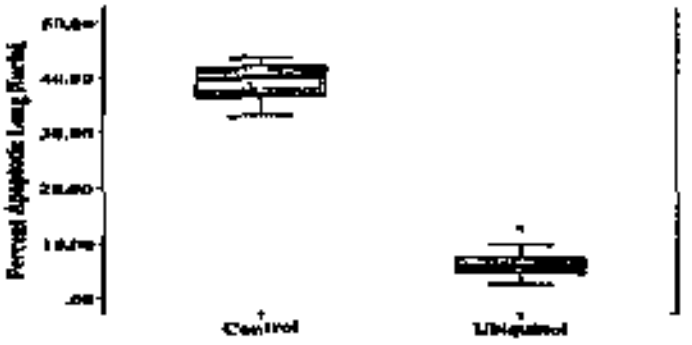
The data indicated that administering IV ubiquinol following hemorrhagic shock facilitated the decrease in the production of superoxide (one of the major reactive oxygen species (ROS)) in the mitochondria. Significant reduction in ROS production in a hemorrhagic shock event could potentially attenuate reperfusion injury induced by fluid resuscitation.

Research Hypothesis 1b: Administering IV CoQ10 reduces lung hydrogen peroxide and apoptosis following HS.

We have completed all the experiments related to IV CoQ10 administration and lung apoptosis following hemorrhagic shock with a sample size of 10 rats in each group. We used fluorescent microscopy to determine nuclear apoptosis.[3] In these experiments, IV CoQ10 significantly decreased the mean percent of lung apoptosis following hemorrhagic shock and fluid resuscitation. These data were published in *Experimental Physiology*, which showed that

rats treated with ubiquinol had significantly less apoptotic nuclei ($p < 0.001$) than the controls in the lungs ($6.0 \pm 0.7\%$ versus $39.2 \pm 1.1\%$) (Figure 2). These findings suggest that administering IV ubiquinol during fluid resuscitation following 60-minute hemorrhagic shock decreases lung injury measured by percent lung apoptosis.

Figure 2. Mean percent lung apoptosis following 60-minute hemorrhagic shock and fluid resuscitation with or without ubiquinol .

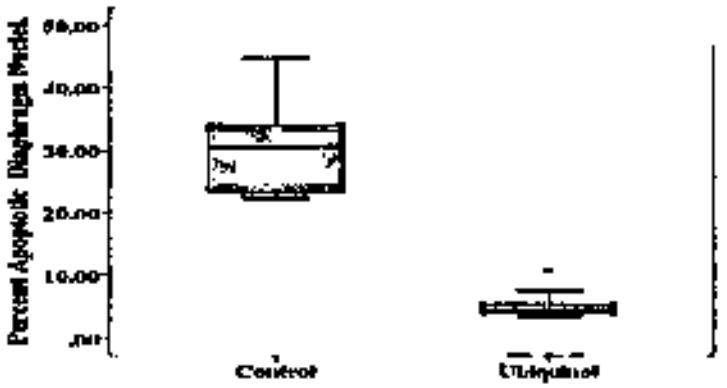


* = significantly different from control ($p < 0.05$). Mean ± SEM, n = 10 per group.

Research Hypothesis 1c: Administering IV CoQ10 reduces diaphragm hydrogen peroxide and apoptosis following HS.

All data collection for this specific hypothesis were completed with 10 experiments in each group (the control and ubiquinolgroups). The rats treated with ubiquinol had significantly less apoptotic nuclei ($p < 0.001$) than the controls in the diaphragm ($4.7 \pm 0.5\%$ versus $30.6 \pm 2.4\%$). The Mann-Whitney U test showed that the mean difference in diaphragm apoptosis between the two groups were significantly different ($p< 0.001$). In other words, administering IV CoQ10 following hemorrhagic shock significantly decreased diaphragm apoptosis by nearly 6 times (See Figure 3 below). Data from this set of experiments indicates that administering IV ubiquinol significantly attenuates diaphragm apoptosis following 60-minutes hemorrhagic shock and fluid resuscitation. These findings were similar with those observed in lung apoptosis.

Figure 3. Mean percent diaphragm apoptosis following 60-minute hemorrhagic shock and fluid resuscitation with or without ubiquinol.



* = significantly different from control ($p < 0.05$). Mean ± SEM, n = 10.

As described in detail in our first annual report, we were unable to use the laser scanning cytometer as planned to measure hydrogen peroxide concentration in the diaphragm due to it being inoperable and personnel change in the flow cytometry center. However, we found an alternative way to achieve this goal, which is laser confocal microscopy. In brief, we did not change any of our methods (used dihydrofluoresecin diacetate (HFluoro-DA) to detect hydrogen peroxide)[4] but only the instrument (from laser scanning cytometer to the laser confocal microscope) to measure hydrogen peroxide. Since then, we continued collaboration with the confocal microscopy expert in the Live Imaging Core Laboratory and have successfully completed all the experiments related to diaphragm hydrogen peroxide production and obtained all the images using a Nikon TE 2000-U inverted three-laser confocal microscope system. We used Metamorph V 7.1.6 software to quantify the average fluorescence intensity of Hfluor-DA from all the fluorescence images. The higher fluorescence intensity measurements, the greater concentration of hydrogen peroxide was in the diaphragm. These data are unchanged from the data presented in the second annual report and are illustrated below. Figure 4 displays examples

of diaphragm sample images captured by a confocal microscope following hemorrhagic shock and fluid resuscitation with and without ubiquinol (shown in Year 1 annual report).

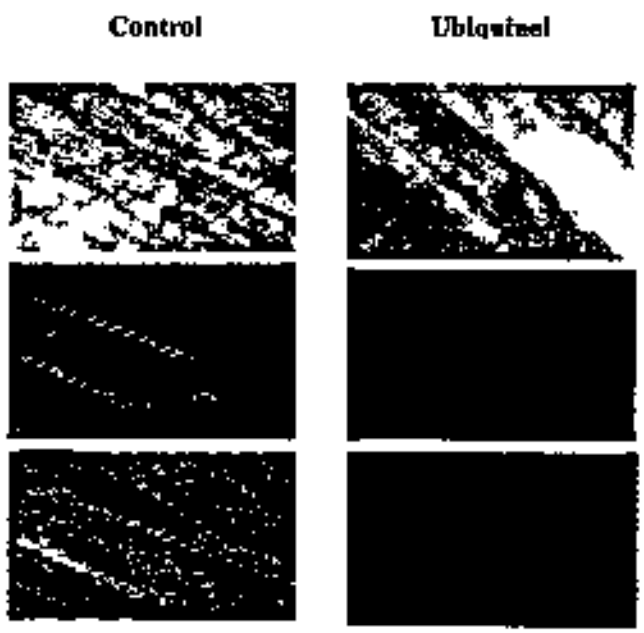
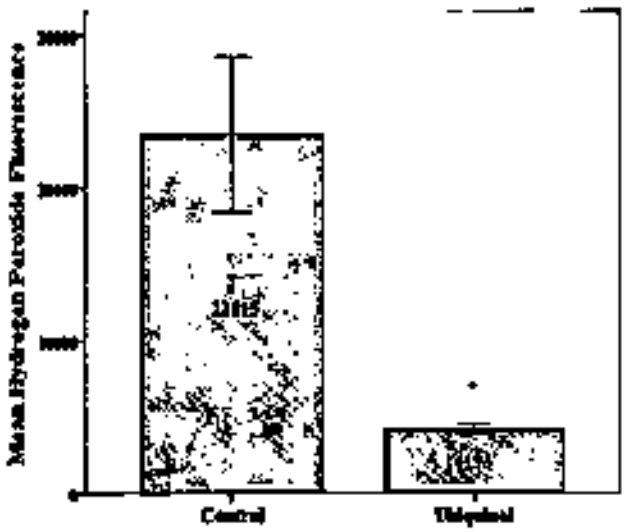


Figure 4. Hydrogen peroxide formation in rat diaphragms following 60 minutes of hemorrhagic shock and fluid resuscitation with & without ubiquinol. A, B, C represent images of a rat diaphragm treated without ubiquinol (A: Confocal bright field, B: Confocal fluorescent image, C: Merged bright field and fluorescent images). D, E, F represent images of a rat diaphragm treated with ubiquinol (D: Confocal bright field, E: Confocal fluorescent image, F: Merged bright field and fluorescent images).

The differences in diaphragm hydrogen peroxide between the two groups were likewise tested using the Mann-Whitney U. Statistical significance was defined as $p \leq 0.05$. Our data showed that the MFI of Hfluor-DA in the control group (23,513 ± 5098) was significantly more than 5 times higher than in the ubiquinol group (4,193 ± 333, $p < 0.001$) (Figure 5). The findings suggest that administering IV ubiquinol following hemorrhagic shock can reduce hydrogen peroxide production in the diaphragm.

Figure 5. Diaphragm mean fluorescent intensity of hydrogen peroxide in the control and ubiquinol group.



* = significantly different from control ($p < 0.05$). Mean ± SEM, n = 8.

In conclusion, all the experiments related to AIM #1 and the three hypotheses suggest that IV CoQ10 reduces the production of ROS (superoxide and hydrogen peroxide) and attenuates cellular injury measured by apoptosis in the lung and diaphragm following hemorrhagic shock and fluid resuscitation. These data have been published in *Experimental Physiology*.[5]

AIM #2: To examine the effect of CoQ10 in the microcirculation as a treatment for HS.

Research Hypothesis 2a: Administering IV CoQ10 decreases leukocyte adherence following HS.

All of the experiments on leukocyte adherence following hemorrhagic shock and fluid resuscitation have been conducted. In the study, leukocyte adherence in the microcirculation was measured on a 100-μm length of mesenteric venule for one minute every 10 minutes throughout the experiment, and recorded on a digital video recorder. Leukocytes adherence was defined as the number of adhered leukocytes in the venule that remained stationary for longer than 30

seconds. Figure 6 (as shown in the second annual report) illustrates the number of leukocyte adherent in mesenteric microcirculation during 60 minutes of hemorrhagic shock and following fluid resuscitation with or without administering IV ubiquinol.

Figure 6. Mean leukocyte adherence during 60-minute hemorrhagic shock and following fluid resuscitation with or without CoQ10.

As seen in Figure 6, leukocyte adherence at baseline was minimal and comparable between the control and ubiquinol groups. During the 60-minute hemorrhagic shock period, the numbers of adherent leukocytes in both groups gradually increased from baseline. However, leukocyte adherences in the control group continued to increase markedly after fluid resuscitation without administering ubiquinol. In contrast, the trend of increased leukocyte adherence during hemorrhagic shock in the ubiquinol group reversed after administering IV ubiquinol along with fluid resuscitation. Furthermore, ubiquinol administration significantly reduced leukocyte adherence to baseline level. The leukocyte adherence after fluid resuscitation treatment in the control group was significantly greater than the CoQ10 group. These data indicate that administering IV CoQ10 can decrease leukocyte adherence in mesenteric microcirculation following hemorrhagic shock and after fluid resuscitation.

Research Hypothesis 2h: Administering IV CoQ10 decreases mast cell degranulation following HS.

Since the last annual report, the experiments related to mast cell degranulation have been completed. The degree of mast cell degranulation was measured by the uptake extent of ruthenium red (an inorganic dye that can stain degranulated mast cells) by mast cells at the end of hemorrhagic shock and fluid resuscitation. At baseline, images of ten intact mast cells surrounding the 100-μm mesenteric venule were obtained for each experiment. At the end of each experiment, the images of the same ten mast cells were collected to compare the degree of mast cell degranulation.

To quantify the degree of mast cell degranulation, all the images were first digitized grayscales and subsequently phase inverted. The relative light intensity of each mast cell within the field of view was measured. The degree of mast cell activation was assessed by calculating the ratio of experimental to baseline light intensities. Figure 7 shows examples of mast cell images that were digitized grayscales and phase inverted.

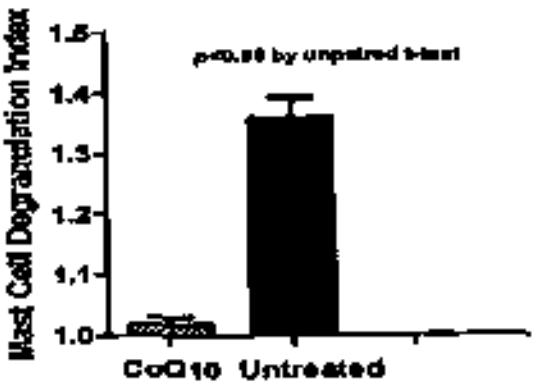
Bright Field

Gray Scale – Phase Inverted

Figure 7. Example of bright field and gray scale - phase inverted mast cell images.

A higher mast cell degranulation index (ratio of experimental to control light intensities) indicates a higher degree of mast cell degranulation. Our data showed that mast cell degranulation index in the control group (1.02 ± 0.01) was significantly higher than that in the ubiquinol group (1.36 ± 0.03, $p < 0.05$) (Figure 8). This suggests that mast cell degranulation in the animals treated with ubiquinol following hemorrhagic shock was significantly less. Corresponding with data on leukocyte adherence, these data indicate that administering IV ubiquinol decreases microcirculation inflammation as reflected by leukocyte adherence and mast cell degranulation.

Figure 8. Mast cell degranulation index between the control and ubiquinol groups.



Research Hypothesis 2et: Administering IV CoQ10 decreases vascular permeability following HS.

After we completed the data collection for leukocyte adherence and mast cell degranulation, we started the experiments related to vascular permeability in microcirculation. We have conducted all of the experiments for this set, with an n of six for each group (control, ubiquinol). To measure vascular permeability, we injected FITC-labeled bovine albumin (50 mg/kg) via femoral artery cannulation 30 minutes before examining mesenteric microcirculation. Using an intensified charge-coupled device (ICCD) camera, fluorescence intensity of FITC-labeled albumin was recorded at an excitation wavelength of 420-490 nm and an emission

wavelength of 520 nm. The duration of each fluorescence recording was less than 15 seconds in a given area. The fluorescence intensity in the selected venule and the surrounding area was measured. The vascular permeability index was calculated as the ratio of extravascular to intravascular fluorescence in intensities. A higher level of vascular permeability index indicates an increase in vascular permeability. Figure 9 demonstrates vascular permeability images of mesenteric venules at baseline, shock, 1 hour, and 2 hours post fluid resuscitation with and without ubiquinol. As seen in the figure, at baseline, vascular permeability was relative low and intravascular fluorescence intensity was much higher (brighter) than extravascular fluorescence intensity.

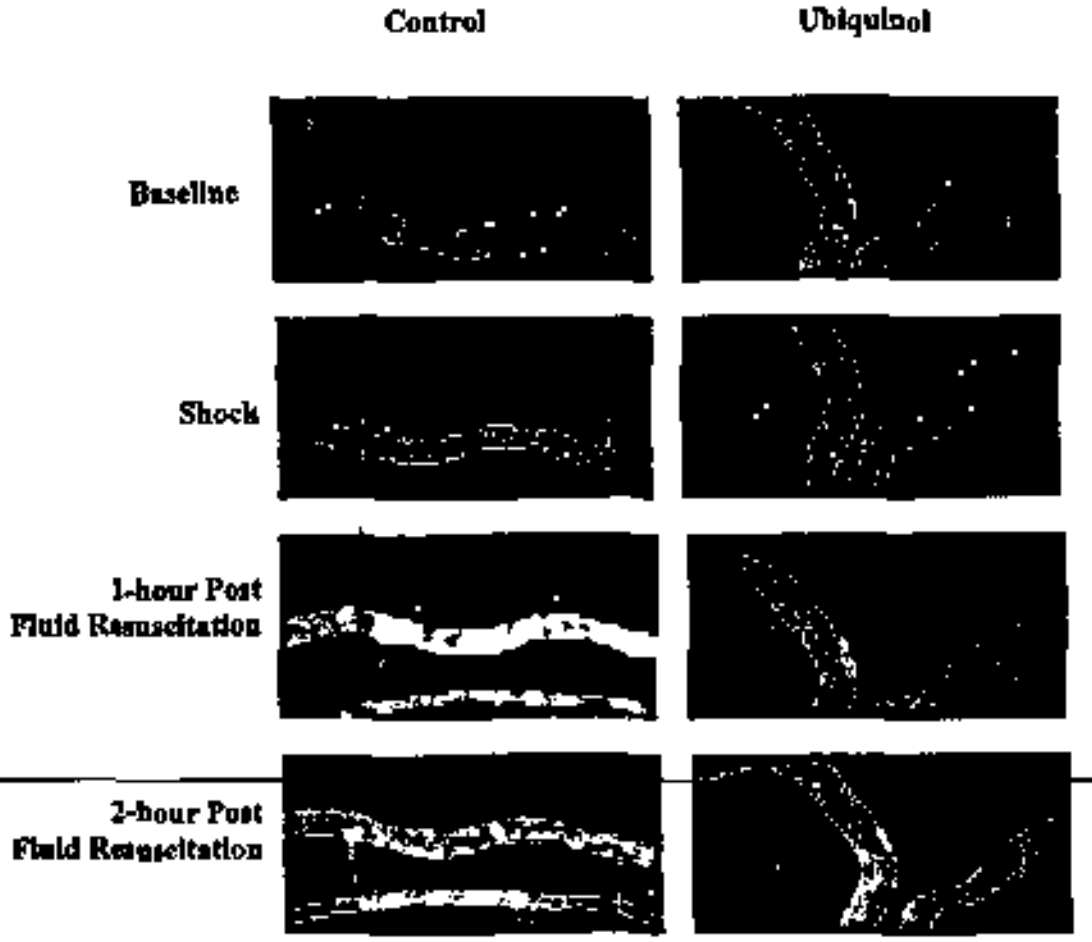


Figure 9. Vascular permeability images of mesenteric venules at baseline, shock, 1 hour, and 2 hours post fluid resuscitation with and without ubiquinol.

Corresponding with other inflammatory responses (leukocyte adherence and mast cell degranulation) to hemorrhagic shock and 1-hour fluid resuscitation, the extravascular fluorescence intensity started increasing as vascular permeability increased, allowing leakage of FITC-labeled bovine albumin from inside of mesenteric venules into extravascular spaces. After 2-hour fluid resuscitation, more FITC-labeled bovine albumin leaked and extravascular fluorescence intensity continued to rise, indicating higher vascular permeability. Table 2 summarizes vascular permeability index, calculated as the ratio of extravascular to the intravascular FITC-albumin fluorescence intensity.

Table 2. Vascular permeability index at baseline, after hemorrhagic shock, lastly 1 and 2 hours after treatment with or without ubiquinol.

Time	Control (n=6)	Ubiquinol (n=6)
Baseline	0.05 ± 0.02	0. 09± 0.01
Shock	0.17 ± 0.06	0.15 ± 0.02
1 Hour Post Treatment	0.36 ± 0.08	0.38 ± 0.09*
2 Hours Post Treatment	0.34 ± 0.02*	0.54 ± 0.05*+

+Significantly different from baseline ($p < 0.05$). *Significantly different from the control group ($p < 0.01$).

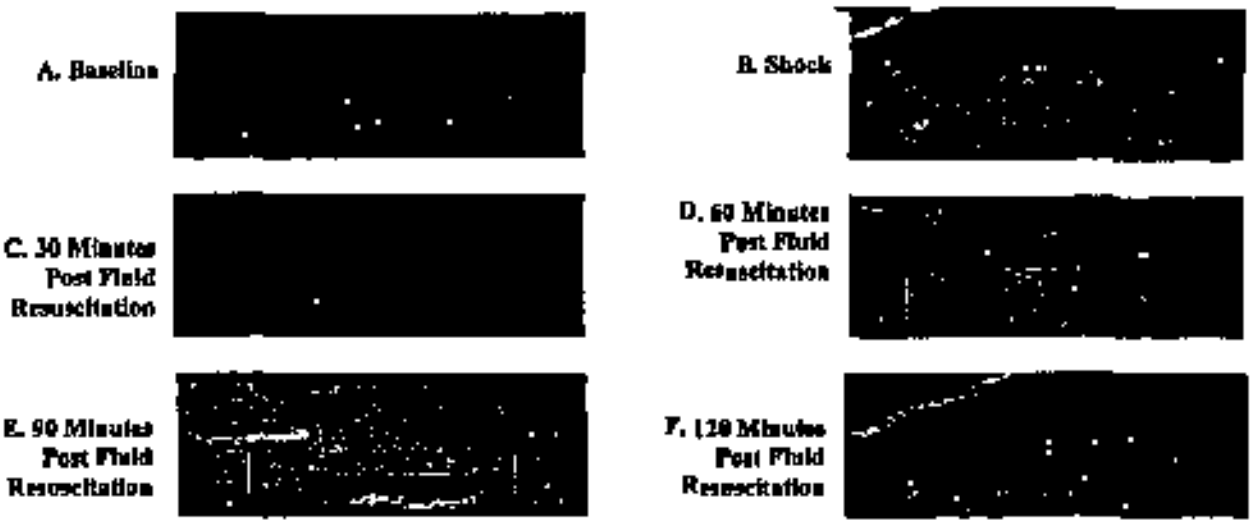
The greater the vascular permeability to FITC-albumin, the higher is the vascular permeability index. At baseline, the vascular permeability indexes in both the control and ubiquinol groups were similar and relatively low. After 1 hour of hemorrhagic shock, the vascular permeability index increased to 0.17 ± 0.06 in the control group and 0.15 ± 0.02 in the ubiquinol group respectively, indicating an increase in vascular permeability. The differences in

vascular permeability index between the control and ubiquinol groups at hemorrhagic shock were not statistically significant ($p > 0.05$). Similar results were observed at 1 hour after treatment in both the control and ubiquinol groups as the vascular permeability indexes continued to increase significantly relative to baseline (0.36 ± 0.08 vs. 0.38 ± 0.09, respectively). Administration of ubiquinol significantly reduced the increase in vascular permeability at 2 hours after treatment to 0.34 ± 0.02, which was significantly less than the control group (0.54 ± 0.05) ($p < 0.01$).

Research Hypothesis 2d: Administering IV CoQ10 decreases microvascular ROS levels following HS.

The experiments for this hypothesis related to microvascular ROS production following hemorrhagic shock were complete. We used the oxidant-sensitive probe called dihydrorhodamine (DHR) to measure ROS levels within mesenteric venules by using an intensified charge-coupled device (ICCD) camera. Recordings of the DHR fluorescence were made for brief intervals (i.e., ~ 15 seconds) to avoid light-induced activation of the probe. The fluorescence intensity was measured during playback of videotapes by using image analysis software (NIH Image 1.62). The fluorescent intensity signals were measured in five adjacent circles of 5-μm diameter along the venular wall. These fluorescent intensity signals were averaged to obtain a single estimate for *fluorescent* signal during each experimental period (baseline, shock, and after fluid resuscitation).

Figure 10. Effects of ubiquinol on DHR fluorescence intensity in the mesenteric microcirculation following hemorrhagic shock and fluid resuscitation. Fluorescent intensity of DHR at baseline, shock, and 30, 60, 90, and 120 minutes after fluid resuscitation in the control and ubiquinol groups.



As seen in Figure above, DHR fluorescence intensity along the venular wall and perivascular sites was minimal at baseline (Figure 10A). After 1-hour hemorrhagic shock, there was slightly increased DHR fluorescence intensity along the venular wall, but not at perivascular sites (Figure 10B). During the first hour of fluid resuscitation, significantly increased DHR fluorescence intensity was noticeable at perivascular sites (Figure 10C and 10D). This trend continued after 90 minutes fluid resuscitation with maximum amount of DHR fluorescence intensity along the venular wall and perivascular sites (Figure 10E). However, CoQ10 decreased DHR fluorescence intensity at all sites slightly after 2 hours fluid resuscitation (Figure 10F). The cumulative data of ROS levels estimated by DHR fluorescence intensity at baseline, HS, 30-min, 60-min, 90-min, and 120-min after fluid resuscitation in the control and ubiquinol groups are displayed in table 3.

Table 3. The fluorescence intensity at baseline, hemorrhagic shock, 30, 60, 90, and 120 minutes after treatment with or without ubiquinol.

Time	Control (n=6)	Ubiquinol (n=6)
Baseline	100	100

Shock	182*	119
30 Minutes Post Treatment	233+	134*
60 Minutes Post Treatment	318+	184*
90 Minutes Post Treatment	335+	199*
120 Minutes Post Treatment	343+	159+*

+Significantly different from baseline ($p < 0.05$). *Significantly different from the control group ($p < 0.01$).

The DHR fluorescence intensities at hemorrhagic shock and every 30 minutes after fluid resuscitation were expressed relative to baseline value (100%). The ROS levels in the control group significantly increased compared to baseline following fluid resuscitation ($p < 0.01$). The ROS levels in the ubiquinol group at 2 hours after fluid resuscitation also significantly increased to 159 ± 35% of baseline ($p < 0.01$); however, it was significantly less than the control group (343 ± 47%) ($p < 0.01$). The data indicated that administering IV ubiquinol along with fluid resuscitation after a hemorrhagic shock event decreased microvascular ROS production. This could potentially reduce microcirculation injury caused by reperfusion after hemorrhagic shock.

In conclusion, all the experiments related to AIM #2 and the four hypotheses suggest that IV CoQ10 reduces leukocyte adherence, mast cell degranulation, vascular permeability, and ROS levels in microcirculation following hemorrhagic shock and fluid resuscitation. These data have been accepted for publication in *Physiological Reports*.[6]

Relationship of current findings to previous findings:

AIM #1: To examine the effect of CoQ10 in leukocytes, the lung, and the diaphragm as a treatment for hemorrhagic shock (HS).

There was significantly less leucocyte mitochondrial O_2^- and diaphragm H_2O_2 in the ubiquinol than the control group. Nuclei of the diaphragm and lungs were measured using fluorescence microscopy. Apoptosis was reduced in the rats treated with ubiquinol. These results suggest that ubiquinol may be protective again organ injury caused from hemorrhagic shock. Oxidative stress can be decreased in numerous diseases with antioxidants.

Antioxidant are present in various compounds and exhibit the possibility to decrease the damaging effects of ischemia–reperfusion injury following hemorrhagic shock and fluid resuscitation.[7,8] Ubiquinol is the only lipid-soluble antioxidant synthesized endogenously.[9] Ubiquinol reduces ROS and assists in creating the proton gradient needed for the rephosphorylation of ADP to ATP in the mitochondrial electron transport chain.[10,11] Ubiquinol sustains the membrane potential and stabilizes permeability transition pores in the mitochondria, as well as controls apoptosis activation.[12] Aoyagi (1984) induced 1 hour of hemorrhagic shock following the administration of ubiquinone and found that the urinary and cardiac outputs in canines significantly recovered better than the control dogs.[13] In another study, ubiquinone did not affect coagulation and suppressed fibrinolysis following hemorrhagic shock.[14] Yamada (1990) induced hemorrhagic shock 60 minutes following the administration with ubiquinone in a dog model.[15] Airway pressures were decreased, lung compliance recovered, plasma pyruvate, catecholamines, histamine, and lactate levels were reduced. Since these studies, our investigations have been the only reported studies of ubiquinol on the generation of ROS or effects on the apoptosis following HS and fluid resuscitation.

Earlier studies suggested that CoQ10 is an antioxidant involved with the mitochondrial ATP generation; these studies are supported by our findings. Cellular hypoxia is produced from hemorrhagic shock and a cascade of events is initiated which leads to the release of inflammatory

cytokines, reduced mitochondrial ATP production, and increased $O_2^{\cdot-}$, H_2O_2 and $OH^{\cdot}$ production.[16] Release of cytokines from the ischemic gut enters the circulation of the vasculature via the mesenteric lymph system following resuscitation and reperfusion. Priming and migration of neutrophils in the heart, kidneys and lungs causing direct local cytotoxic injury by the release of pro-inflammatory mediators and ROS. Hypoxic conditions cause an increase in cardiac mitochondria ROS (Kalamunne et al., 2011). Similarly, our results showed that hemorrhagic shock induced hypoxia and the production of ROS, this was measured by using MitoSOX Red.[17,18] Administering ubiquinol post hemorrhagic shock decreased mitochondrial $O_2^{\cdot-}$ ensuing the preservation of mitochondrial membrane integrity and electron transport chain complex activity. Cellular apoptosis in reperfusion injury occurs from the release of ROS and inflammatory mediators. Cellular oxygen increases when blood flow returns following fluid resuscitation, causing an increase in xanthine dehydrogenase production, resulting in the increase of $O_2^{\cdot-}$ and H_2O_2 production.[19]

In our study, we used blood and lactated Ringer solution to restore blood flow, this was followed by a decrease of hypoxic cells and this increased free radical production that could damage proteins, DNA, and plasma membranes. An increase in leukocyte mitochondrial $O_2^{\cdot-}$ and H_2O_2 concentration in the diaphragm occurred at 120 minutes after fluid resuscitation without using ubiquinol. Lower levels of $O_2^{\cdot-}$ and H_2O_2 were a result of the scavenged ROS that occurred when ubiquinol was used. Reactive oxygen species activated phagocytes are mediators of apoptotic and necrotic organ injury.[20] The generation of $O_2^{\cdot-}$ is possibly a result of the involvement of leukocyte mitochondria.[21] In our study, the MFI of MitoSOX Red using ubiquinol significantly decreased 120 minutes following hemorrhagic shock, the MFI without using ubiquinol continued to increase. The study suggest that ubiquinol scavenges leukocyte

mitochondrial $O_2^{\cdot-}$ when it was found that $O_2^{\cdot-}$ decreased following fluid resuscitation in the ubiquinol group compared with the control group. The oxidant, H_2O_2 is involved in the physiology and signaling of cells. In one study following hemorrhagic shock, dopamine decreased the level of H_2O_2 in rat diaphragms.[7] In aim #1, the concentration of H_2O_2 in the diaphragms were significantly less in the animals that were administered ubiquinol, when compared with the control group. Diaphragmatic ROS could possibly be decreased if ubiquinol is used post-hemorrhagic shock before resuscitation with fluid, contractile function would recover and respiratory impairment risk decrease. It is well known that there is a correlation with increased ROS and apoptosis.[20] Organ damage and apoptosis are associated with hemorrhagic shock and injury from reperfusion. Apoptosis is decreased following antioxidant administration in an animal hemorrhagic shock model.[22] Hydrogen peroxide production corresponded with the reduction of antioxidants in apoptotic cells.[23] In the late stages of hemorrhagic shock, post hypoxic-reperfusion, raised levels of ROS in organs cause damage to the mitochondria that can lead to apoptosis and result in organ damage and eventually failure. It was concluded by Kolamunne et al. (2011) that a higher $O_2^{\cdot-}$ exposure was the cause of cell death after noticing a decrease of $O_2^{\cdot-}$ in hypoxic cardiac myoblasts. Our aim #1 findings were similar to Kolamunne in that there was not a significant increase in the ubiquinol group for presence of $O_2^{\cdot-}$ in the mitochondria, with a corresponding low percent apoptosis. In addition, it was found that the use of ubiquinol pre resuscitation reduced diaphragm and lung apoptosis, suggesting ubiquinol has antioxidant properties. The attenuation of apoptosis activation results from a decrease in ROS.

The data for aim #1 suggest ubiquinol may useful as a supplemental treatment for hemorrhagic shock and resuscitation injury due to the following: A reduction of leucocyte $O_2^{\cdot-}$ in

the mitochondria, a decrease in H_2O_2 concentration in the diaphragm, and reduced lung and diaphragm apoptosis.

AIM #2: To examine the effect of CoQ10 in the microcirculation as a treatment for HS.

The administration of ubiquinol following hemorrhagic shock and fluid resuscitation has the following effects on mesenteric venules: leukocyte adherence reduction, mast cell degranulation attenuation, and lessens the vascular permeability and ROS level increases.

The balance between pro-adhesive and hydrodynamic dispersal forces are the determining factors affecting leukocyte adherence to the venular wall. There are a variety of pro inflammatory mediators that are pro adhesive forces such as activating factor (PAF), histamine, platelets, and leukotrienes which are involved in cells of the endothelium and leukocyte receptors, stimulating rolling and adhesion of the leukocytes.[24] Activated endothelial cells caused from a raised level of ROS and contribute to transcription-dependent adhesion molecule synthesis and expression.[25,26] An anti-adhesive force is the movement of blood flow, which propels leukocytes around the endothelial surface. Venular wall shear rate is the estimation representing the leukocyte adherence interference caused from the hydrodynamic dispersal force generated at the vessel wall. During no time throughout the experiments were there any significant differences between the control and ubiquinol groups for shear rate. The differences between the groups for adherent leukocytes might be the result of pro-adhesive force differences.

Reactive oxygen species reduction in the microcirculation of the mesentery occurs from the antioxidant, ubiquinol, therefore the transcription-dependent adhesion molecule synthesis and expression may be inhibited. Platelet activating factor (PAF) and leukotrienes are important inflammatory mediators that have crucial development function in microvascular inflammatory response to hemorrhagic shock and fluid resuscitation induced ischemia-reperfusion.[27]

Adherence of leukocytes stimulated by the release of PAF also increased leukocyte superoxide production,[26] superoxide dismutase stops PAF generated adherence of leukocytes.[28] In our study, we found that administering ubiquinol prior to fluid resuscitation may reduce PAF induced leukocyte adherence by lowering levels of ROS. In addition, ubiquinol may decrease mast cell degranulation post 120 minutes of fluid resuscitation. Various inflammatory mediators (i.e., serotonin, kinogenases, histamine, tryptase, phospholipases, etc.) are released during mast cell degranulation as well as strengthen the inflammatory responses in the microcirculation.[29]

Reperfusion injury that occurs post hemorrhagic shock and treatment with fluid resuscitation can be minimized by hindering degranulation of the mast cells. A mast cell stabilizer by the name of cromolyn and the antioxidant known as lipoic acid was detected to block degranulation of mast cells, thus decreasing ROS, vascular permeability, and adherence of leukocytes.[30] Blood flow and decreased hemoglobin concentration was most likely the cause of mesenteric hypoxia. Other studies indicated that if homeostasis does not occur between ROS and nitric oxide, mast cell degranulation may be initiated and an increased adhesion of leukocytes to the endothelium.[31,32] In our studies, it is possible that ubiquinol is responsible for decreasing ROS levels and degranulation of mast cells. Comparable to other studies, ischemia-reperfusion stimulated vascular permeability to FITC-albumin in the control group.[33,34] Varied FITC-albumin patterns accrued along the venule walls, suggesting leakage in the vasculature following hemorrhagic shock and fluid resuscitation.

Histamine and serotonin inflammatory mediator activation and vascular endothelial growth factor (VEGF) cause changes in the vascular ultrastructure and increase permeability.[35] Changes in the vascular integrity occur from the involvement of ROS.[36] Reactive oxygen species are scavenged by ubiquinol, when this happens vascular permeability is decreased by a reduction

in histamine and serotonin release by mast cells. In our study, ubiquinol administered post hemorrhagic shock decreased ROS in the venular wall of the mesentery. When ubiquinol was not given, an increase in ROS occurred during the period of 120 minutes following fluid resuscitation, thus causing harm to the microcirculation.

Microvascular inflammation and degranulation of mast cells are caused by a release of inflammatory mediators into the circulation from activation of alveolar macrophages during the hypoxic conditions that occur from hemorrhagic shock and fluid resuscitation.[37] Endothelial cell activation and production of ROS increases, as well as a decrease in nitric oxide occur during this time of ischemia/reperfusion.[38] The nitric oxide and ROS imbalance that happens during reperfusion increases increased degranulation of mast cells, adherent leukocytes, and vascular permeability in the control group, or reperfusion injury.

In our study, administering ubiquinol attenuated ROS production that results post fluid resuscitation, thus sustaining mast cell strength, decreasing leukocyte adhesive interactions in the endothelium, and vascular permeability reduction. The data for aim #2 concludes that ubiquinol protect organs and circulation of the microvasculature from the injury that happens because of reperfusion with fluids following hemorrhagic shock.

Effect of problems or obstacles on the results:

As reported in the last annual report for Year 2, we encountered some challenges with the experiments related to vascular permeability and microcirculation ROS production. The microscope that was used for all microcirculation experiments has two light source channels with two different cameras: bright field and fluorescence. We used the bright field camera for the mast cell degranulation experiments. Use of FITC-labeled bovine albumin in the vascular permeability experiments requires switching the bright field camera to fluorescence. However,

for the first two experiments, the switch failed to successfully alternate to the fluorescence camera. We solved this problem by obtaining assistance from a microscopy specialist from the Imaging Center at University of Kansas Medical Center. He tested the microscope and found a way in which we could switch the cameras manually. Therefore, we were able to visualize the venules by carefully switching the cameras manually for each microcirculation experiment. The results were not affected by this problem.

Identifying the optimal concentration of FITC-labeled bovine used for arterial injection was challenging. At the beginning, a higher concentration of FITC-labeled bovine albumin was used and the intravascular fluorescence intensity was too bright and caused an automatic shut off by the fluorescence camera. We tested different concentrations to obtain better fluorescence contrast between intravascular and extravascular spaces and had found that 50 mg/kg is an appropriate dose. Since then, we have been successfully obtaining quality of fluorescence images to evaluate vascular permeability.

During the process of converting and analyzing images from the first few mast cell degranulation experiments, we found that the gray scale-phase inverted images were too dark to detect any light intensity. We consulted with microcirculation specialist Dr. Wood and he suggested washing off the super-perfused ruthenium red solution on mesentery prior to recording images. Since we tested and applied this method to wash off excessive ruthenium red solution covering the mesentery, we have been able to measure light intensity of mast cell to evaluate its degree of degranulation.

With vascular permeability studies it was difficult on some days to find mesenteric that had sufficient flow or did not have bifurcations. To overcome these difficulties, we spent more time searching the various venules and it sometimes required up to an hour or longer to find an

adequate vessel. Thus, adding additional time for searching for venules assisted us with overcoming this obstacle.

The major obstacles with the ROS experiments was performing these experiments in the dark and remembering to add additional DHR to the venule to ensure there was adequate dye for ROS measurements. We overcame these obstacles by using timers to remember to add the DHR solution and using our smart phones with flashlights to assist with data recording and to visualize our catheters when needed.

Limitations:

There are three major limitations of this study. First, these finding are in rats and would not be generalizable to humans. Thus, there will need to be translational studies in humans before this can be implemented in practice. The second limitation is the use of intravenous (IV) ubiquinol in humans. In Europe, Japan and China ubiquinol is available in an IV form. However, in the U.S., it is currently not available to give IV and there would need to be approval before using ubiquinol in this formulation. The third limitation is the requirement for immediate measurement of $O_2^{\cdot-}$ using a flow cytometer. This requires reservation of the instrument and the time available to test the sample, which is not always possible.

Conclusion:

The completed experiments related to AIM # 1 suggests that administering IV CoQ10 following hemorrhagic shock and during fluid resuscitation has significant effects on reducing the production of reactive oxygen species (hydrogen peroxide and superoxide) in the lung, diaphragm, and mitochondria in leukocytes. In addition, IV CoQ10 significantly reduces cellular injury (measured by percent of apoptosis) in the vital organs such as the lungs and diaphragm. Our microcirculation data for AIM #2 also indicate that adding IV CoQ10 to the fluid

resuscitation regimen following hemorrhagic shock effectively decreases leukocyte adherence, mast cell degranulation, vascular permeability, and microvascular ROS. This suggests that adding IV CoQ10 could substantially reduce microcirculation inflammation and injury that occurs with reperfusion injury. Therefore, administering CoQ10 following hemorrhagic shock may help prevent cellular damage caused by hemorrhagic shock that often leads to the organ failure. The data for AIM #1 have been successfully disseminated in presentations and published in *Experimental Physiology*. The data for AIM #2 have been successfully disseminated in presentations and have been accepted for publication in *Physiological Reports*.

Significance of Study or Project Results to Military Nursing

Many military personnel in the recent Operations in Iraq and Afghanistan have experienced hemorrhagic shock.[39] With most hemorrhage-related deaths occurring 6 hours post-injury, it should be a priority for military nurses to establish an efficient mode of treatment in order to improve survival rates among war-fighters and other operational personnel. Military and civilian personnel need research evidence to base their practice related to resuscitation therapy in hemorrhagic shock patients. Since resuscitation can result in cellular injury and multiple organ failure, it is essential to determine the optimal mode of treatment that limits lung, diaphragm, and microvasculature cellular damage. With U.S. casualties originating in Iraq and Afghanistan, there is a renewed sense of urgency to find the best method of recovery for military personnel following hemorrhagic events. While current practices are lifesaving, they may not always restore normal cellular and organ function.

The results from this study are significant to military nursing because the findings show a decrease in cellular damage and organ damage as measured by leukocyte presence in the blood, lung and diaphragm apoptosis, and diaphragm hydrogen peroxide concentration. In addition, the damage to the microcirculation as measured by leukocyte adherence, mast cell degranulation, vascular permeability, and reactive oxygen species presence all improved with the addition of the supplement ubiquinol to the hemorrhagic shock treatment protocol. The results support previous suggestions of ubiquinol being a free radical scavenger, a decrease in oxidative stress that is produced following hemorrhagic shock. Our experiments that were conducted with an animal models puts military nursing one-step closer to beginning translational processes in the field, possibly increasing the survival for the wounded warriors. The results of these experiments have the potential to change practice by providing knowledge needed for measuring patient outcomes

experiencing free radical damage following a hemorrhagic event. Our study examined factors that affect the health care of operational personnel that experience hemorrhagic shock. The results of this study indicate ubiquinol can be used a potential treatment for hemorrhagic shock and lead to improving health outcomes for those we care for that have life threatening conditions, resulting in better care for the war-fighter.

The results indicate that CoQ10 can be a chemical adjunct to current resuscitation treatments that may ultimately work to reduce the damaging effects of HS and resuscitation. The results of this study reveal that using a blood test for the presences of leukocytes may act as a biomarker for oxidative stress during hemorrhagic shock, and the administration of ubiquinol added to the protocol to decrease the oxidative stress and accompanying cellular damage and organ death. The possibility of reducing cellular injury can prolong life and promote health of trauma patients, military and civilian. For military nurses, hemorrhagic shock occurs in many life-threatening situations. If a more optimal mode of therapy for hemorrhagic shock were established, such as the addition of ubiquinol, it would allow military nurses to provide care that is more efficient for the warrior and other military personnel.

Military nursing clinical practice could benefit from the results of these studies should the guidelines for hemorrhagic shock resuscitation includes the addition of CoQ10 used to prevent multiple organ damage by decreasing oxidative stress. Our data expands the body of military-relevant scientific knowledge and ultimately improve the ability to provide proper and high-quality care for operational personnel who suffer from hemorrhagic shock. Lastly, the results of our study indicate that caring for all entrusted to our care could lead to improving health outcomes for those we care for that are in life threatening situations, resulting in better care for the war fighter.

Changes in Clinical Practice, Leadership, Management, Education, Policy, and/or Military Doctrine that Resulted from Study or Project

None to date.

References Cited

1. Mukhopadhyay P., Rajesh M., Yoshihiro K., Hasko G., & Pacher P. (2007). Simple quantitative detection of mitochondrial superoxide production in live cells. *Biochem Biophys Res Commun. 358*(1):203-208. PMC2228267

2. Mukhopadhyay P., Rajesh M., Hasko G., Hawkins B., Madesh M., & Pacher P. (2007). Simultaneous detection of apoptosis and mitochondrial superoxide production in live cells by flow cytometry and confocal microscopy. *Nat Protoc.* 2(9): 2295-2301. PMC2225540

3. Goodyear-Bruch C., Simon K., Hall S., Mayo M., & Pierce, J. (2005). Comparison of a visual to a computer-assisted technique for detecting apoptosis. *Biol Res Nurs.* 6(3):180-186.

4. Zuo, L. & Clanton, T. (2005). Reactive oxygen species formation in the transition to hypoxia in skeletal muscle. *Am J Physiol Cell Physiol. 289*(1): C207-216.

5. Bennetts, P., Shen, Q., Thimmesch, A., Diaz, F., Clancy, R., & Pierce, J. (2014). Effects of ubiquinol with fluid resuscitation following hemorrhagic shock on rat lungs, diaphragm, heart and Kidneys. *Exp Physiol. 99*(7): 1007-1015.

6. Shen, Q., Holloway, N., Thimmesch, A., Wood, J., Clancy, R., & Pierce, J. (2014). Ubiquinol Decreases Hemorrhagic Shock/Resuscitation Induced Microvascular/Inflammation in the Rat Mesenteric Microcirculation. *Physiological Reports.* In Press.

7. Pierce J., Knight A., Slusser J., Gajewski B. & Clancy R. (2011). Effects of fluid resuscitation and dopamine on diaphragm performance, hydrogen peroxide, and apoptosis following hemorrhagic shock in a rat model. *Mil. Med. 176*(3): 336-342.

8. Saad, K., Saad, P., Dantas Filho, L., Brito, J., Koike, M., Zanoni, F., Dolhnikoff, M. & Montero, E. (2013). Pulmonary impact of N-acetylcysteine in a controlled *hemorrhagic* shock model in rats. *J. Surg. Res. 182*(1): 108-115.

9. Bentinger, M., Tekle, M. & Dallner, G. (2010). Coenzyme Q--biosynthesis and functions. *Biochem.Biophys. Res. Commun. 396*(1): 74-79.

10. Villalba, J., Parrado, C., Santos-Gonzalez, M. & Alcain, F. (2010). Therapeutic use of coenzyme Q(10) and coenzyme Q(10)-related compounds and formulations. *Expert Opin Inv Drug 19*(4): 535-554.

11. Littarru, G. & Tiano, L. (2007). Bioenergetic and antioxidant properties of coenzyme Q10: recent developments. *Mol. Biotechnol. 37*(1): 31-37.

12. Cleren, C., Yang, L., Lorenzo, B., Calingasan, N., Schomer, A., Sireci, A., Wille, E., & Beal, M. (2008). Therapeutic effects of coenzyme Q10 (CoQ10) and reduced CoQ10 in the MPTP model of Parkinsonism. *J. Neurochem. 104*(6): 1613-1621.

13. Aoyagi, N. (1984). Effects of coenzyme Q10 on cardiovascular and endocrine functions during haemorrhagic shock in dogs. *Masui. 33*(5): 493-504.

14. Hatano, K. (1985). Effects of various drugs and fluid on the blood coagulation- fibrinolysis and kinin systems in canine hemorrhagic shock. *Masui. 34*(5): 637-648.

15. Yamada, M. (1990). Effects of coenzyme Q10 in hemorrhagic shock. *Crit. Care Med. 18*(5): 509-514.

16. Knight, A., Fry, L., Clancy, R., & Pierce, J. (2011). Understanding the effects of oxygen administration in haemorrhagic shock. *Nurs. Crit. Care 16*(1): 28-35.

17. Kolamunne, R., Clare, M., & Griffiths, H. (2011a). Mitochondrial superoxide anion radicals mediate induction of apoptosis in cardiac myoblasts exposed to chronic hypoxia. *Arch Biochem. Biophys. 505*(2): 256-265.

18. Kolamunne, R., Clare, M., & Griffiths, H. (2011b). Mitochondrial superoxide anion radicals mediate induction of apoptosis in cardiac myoblasts exposed to chronic hypoxia. *Arch. Biochem. Biophys. 505*(2): 256-265.

19. Ng, C., Wan, S., & Yim, A. (2005). Pulmonary ischaemia-reperfusion injury: role of apoptosis. *Eur. Respir. J. 25*(2): 356-363.

20. Jernigan, T., Croce, M., & Fabian, T. (2004). Apoptosis and necrosis in the development of acute lung injury after hemorrhagic shock. *Am. Surg. 70*(12): 1094-1098.

21. Fossati, G., Moulding, D., Spiller, D., Moots, R., White, M., & Edwards, S. (2003). The mitochondrial network of human neutrophils: Role in chemotaxis, phagocytosis, respiratory burst activation, and commitment to apoptosis. *J. Immunol. 170*(40): 1964-1972.

22. Yang, R., Vernon, K., Thomas, A., Morrison, D., Qureshi, N., & Van Way, C., 3rd (2011). Crocetin reduces activation of hepatic apoptotic pathways and improves survival in experimental hemorrhagic shock. *JPEN. J. Parenter. Enteral Nutr. 35*(1): 107-113. PMC3990265

23. Takayanagi, I., Konno, F., Akaike, M., Nibori, Y., & Yamura, S. (1987). cis(-)-2,3-Dihydro-3-(4-methylpiperazinylmethyl)-2-phenyl-1,5- benzothiazepin-4(5H)-one monohydrochloride and its butylbromide as M1-receptor antagonists. *Gen. Pharmacol. 18*(1): 91-93.

24. Harris, N., Whitt, S., Zilberberg, J., Alexander, J., & Rumbaut, R. (2002). Extravascular transport of fluorescently labeled albumins in the rat mesentery. *Microcirculation* 9(3): 177-187.

25. Granger, D. & Senchenkova, E. (2010). Leukocyte-endothelial cell adhesion. In: *Inflammation and the Microcirculation.* Morgan & Claypool Life Sciences, http://www.ncbi.nlm.nih.gov/books/NBK53380/.

26. Park, T., Gonzales, E., & Gidday, J. (1999). Platelet-activating factor mediates ischemia-induced leukocyte-endothelial adherence in newborn pig brain. *J Cereb Blood Flow Metab 19*(4): 417-424.

27. Bitencourt, C., Bessi, V., Huynh, D., Menard, L., Lefebvre, J., Levesque, T., Hamdan, L., Sobouhenou, F., Faccioli, L., Borgeat, P., & Marleau, S. (2013). Cooperative role of endogenous leucotrienes and platelet-activating factor in ischaemia-reperfusion-mediated tissue injury. *J Cell Mol Med 17*(12): 1554-1565.

28. Steiner, D., Gonzalez, N., & Wood, J. (2002). Interaction between reactive oxygen species and nitric oxide in the microvascular response to systemic hypoxia. *J Appl Physiol 93*(4): 1411-1418.

29. Wood, J., Johnson, J., Mattioli, L., & Gonzalez, N. (1999). Systemic hypoxia promotes leukocyte-endothelial adherence via reactive oxidant generation. *J Appl Physiol 87*(5): 1734-1740.

30. Swindle, E. & Metcalfe, D. (2007). The role of reactive oxygen species and nitric oxide in mast cell-dependent inflammatory processes. *Immunol Rev* 217: 186-205.

31. Granger, D., & Kubes, P. (1994). The microcirculation and inflammation: modulation of leukocyte-endothelial cell adhesion. *J Leukoc Biol 55*(5): 662-675.

32. Theoharides, T., Alysandratos, K., Angelidou, A., Delivanis, D., Sismanopoulos, N., Zhang, B., Asadi, S., Vasiadi, M., Weng, Z., Miniati, A., & Kalogeromitros, D. (2012). Mast cells and inflammation. *Biochim Biophys Acta 1822*(1): 21-33.

33. Castillan, A., Gonzalez, N., Johnson, J., Steiner, D., & Wood, J. (2003). Mesenteric microvascular inflammatory responses to systemic hypoxia are mediated by PAF and LTB4. *J Appl Physiol 94*(6): 2313-2322.

34. Hatano, K. (1985). Effects of various drugs and fluid on the blood coagulation- fibrinolysis and kinin systems in canine hemorrhagic shock. *Masui* 34: 637-648.

35. Bates, D. (2010). Vascular endothelial growth factors and vascular permeability. *Cardiovasc Res* 87: 262-271.

36. Panes, J., & Granger, D. (1998). Leukocyte-endothelial cell interactions: molecular mechanisms and implications in gastrointestinal disease. *Gastroenterology 114*(5): 1066-1090.

37. Childs, E., Udobi, K., Wood, J., Hunter, F., Smalley, D., & Cheung, L. (2002). In vivo visualization of reactive oxidants and leukocyte-endothelial adherence following hemorrhagic shock. *Shock 18*(5): 423-427.

38. Steiner, D., Gonzalez, N., & Wood, J. (2003). Mast cells mediate the microvascular inflammatory response to systemic hypoxia. *J Appl Physiol 94*(1): 325-334.

39. Kisat, M., Morrison, J.J., Hashmi, Z.G., Efron, D.T., Rasmussen, T.E., & Haider, A.H (2013) Epidemiology and outcomes of non-compressible torso hemorrhage. *J Surg Res*.184(1): 414-21. doi: 10.1016/j.jss.2013.05.099. PMID: 23831230

Summary of Dissemination

Type of Dissemination	Citation	Date and Source of Approval for Public Release
Publications	Shen, Q., Knowles, E., Hiebert, J., & Pierce, J. (2012) Mitochondrial health – Essential information for nurses. *Journal of Nursing Education and Practice*, 2(2), 162-170.	15 NOV 2011 John P. Maye, CRNA, PhD, CAPT, NC, USN Executive Director, TSNRP
	Pierce, J., McCabe, S., White, N., & Clancy, R. (2012) Biomarkers: An important assessment tool for clinical science. *American Journal of Nursing*, *119*(9),52-58.	14 DEC 2011 John P. Maye, CRNA, PhD, CAPT, NC, USN Executive Director, TSNRP
	Lance, J., McCabe, S., Clancy, R., & Pierce, J. (2012) Coenzyme Q10 – A therapeutic agent. *MedSurg Nursing: The Journal of Adult Health*, *2*(6), 367-371.	14 DEC 2011 John P. Maye, CRNA, PhD, CAPT, NC, USN Executive Director, TSNRP
	Hiebert, J. B., Shen, Q., & Pierce, J. D. (2012) Application of coenzyme Q10 in clinical practice. *The Internet Journal of Internal Medicine*, *9*(2). http://www.ispub.com/journal/the-internet-journal-of-internal-medicine/volume-9-issue-2/43564399application-of-coenzyme-q10-in-clinical-practive.html.	13 MAR 2012 John P. Maye, CRNA, PhD, CAPT, NC, USN Executive Director, TSNRP
	Brandmeyer, E.A., Shen, Q., Thimmesch, A.R., & Pierce, J. (2014) Use of coenzyme Q10 in clinical practice. *Nursing2014*, *44*(3), 63-66.	16 AUG 2013 Michael Schlicher, PhD, RN, LTC, AN Executive Director, TSNRP
	Bennetts, P., Shen, Q., Thimmesch, A., Diaz, F., Clancy, R., & Pierce, J. (2014) Effects of ubiquinol with fluid resuscitation following haemorrhagic shock on rat lungs, diaphragm, heart and kidneys. *Experimental Physiology*, *99*(7), 1007-1015.	28 MAY 2014 Michael Schlicher, PhD, RN, LTC, AN Executive Director, TSNRP

Publications in Press	Shen, Q., Holloway, N., Thimmesch, A., Wood, J., Clancy, R., & Pierce, J. (2014). Ubiquinol Decreases Hemorrhagic Shock/Resuscitation Induced Microvascular Inflammation in the Rat Mesenteric Microcirculation. *Physiological Reports*	3 SEPT 2014 Michael Schlicher, PhD, RN, LTC, AN Executive Director, TSNRP
	Pierce, J., Shen, Q., & Thimmesch, A. (2014). The Ongoing Controversy: Crystalloids Versus Colloids. *Journal of Infusion Nursing*	3 SEPT 2014 Michael Schlicher, PhD, RN, LTC, AN Executive Director, TSNRP
Published Abstracts	Shen, Q., Bennetts, P., Hastings, R., Zeiger, A., & Pierce, J.D. (2012). Measuring leukocyte mitochondrial superoxide using flow cytometry. State of Science Congress on Nursing Research Conference by the Council for the Advancement of Nursing Science. Washington, D.C. 13-15 SEP 2012.	14 MAY 2012 John P. Maye, CRNA, PhD, CAPT, NC, USN Executive Director, TSNRP
	Shen, Q., Hiebert, J., Pierce, J., Clancy, R., Pierce, J. (2012). Reduction of myocyte hydrogen peroxide and mitochondrial damage in rats following hemorrhagic shock and fluid resuscitation with ubiquinol (Abstract). Critical Care Medicine, 40 (12, Suppl.), 550. DOI: 10.1097/01.ccm.0000424768.17040.ee	09 OCT 2012 John P. Maye, CRNA, PhD, CAPT, NC, USN Executive Director, TSNRP
	Bennetts, P., Shen, Q., Thimmesch, A., Clancy, R., Pierce, J. (2012) Effects of ubiquinol on reactive oxygen species and cellular injury in rats following hemorrhagic shock and fluid resuscitation (Abstract). Critical Care Medicine, 40 (12, Suppl.), 538. DOI: 10.1097/01.ccm.0000424756.94084.cb	09 OCT 2012 John P. Maye, CRNA, PhD, CAPT, NC, USN Executive Director, TSNRP

Podium Presentations	Pierce, J. Effects of CoQ10 on Hemorrhagic Shock. Sigma Theta Tau Conference, Kansas City, KS, 06 NOV 2012	05 NOV 2012 John P. Maye, CRNA, PhD, CAPT, NC, USN Executive Director, TSNRP
	Pierce, J. & Shen, Q. Use of Ubiquinol as a Treatment following Hemorrhagic Shock. University of Kansas, Department of Dietetics and Nutrition. Kansas City, KS, 04 MAR 2013	15 MAR 2013 John P. Maye, CRNA, PhD, CAPT, NC, USN Executive Director, TSNRP
	Pierce, J., Shen, Q., Bennetts, P., Thimmesch, A., & Clancy, R. Using Ubiquinol to Reduce Cellular Injury in Rats Following Hemorrhagic Shock and Fluid Resuscitation. 1st Annual TriService Nursing Research Program Research and EBP Dissemination Course, San Antonio, TX. September 15-18, 2014.	16 SEP 2014 No permission needed. It was a TSNRP function.
	Shen, Q., Holloway, N., Thimmesch, A., Wood, J., Clancy, R., & Pierce, J. Ubiquinol Decreases Hemorrhagic Shock/Resuscitation Induced Microvascular Inflammation in the Rat Mesenteric Microcirculation. Council for the Advancement of Nursing Science, Washington, DC. October 15-18, 2014.	24 MAR 2014 Michael Schlicher, PhD, RN, LTC, AN Executive Director, TSNRP

Poster Presentations	Shen, Q., Hiebert, J. Pierce, T., Clancy, R., & Pierce, J. Reduction of Myocyte Hydrogen Peroxide and Mitochondrial Damage in Rats Following Hemorrhagic Shock and Fluid Resuscitation with Ubiquinol. Society of Critical Care Medicine's 42nd Annual Critical Care Congress. Puerto Rico, USA. 21 JAN 2013.	09 OCT 2012 John P. Maye, CRNA, PhD, CAPT, NC, USN Executive Director, TSNRP
	Bennetts, P., Shen, Q., Thimmesch, A., Clancy, R., Pierce, J. (2012) Effects of ubiquinol on reactive oxygen species and cellular injury in rats following hemorrhagic shock and fluid resuscitation. Society of Critical Care Medicine's 42nd Annual Critical Care Congress. Puerto Rico, USA. 21 JAN 2013.	09 OCT 2012 John P. Maye, CRNA, PhD, CAPT, NC, USN Executive Director, TSNRP
	Shen, Q., Hiebert, J. Pierce, T., Clancy, R., & Pierce, J. Reduction of Myocyte Hydrogen Peroxide and Mitochondrial Damage in Rats Following Hemorrhagic Shock and Fluid Resuscitation with Ubiquinol. University of Kansas, Postdoctoral Research Day, Lawrence, KS. 15 MAR 2013.	9 OCT 2012 John P. Maye, CRNA, PhD, CAPT, NC, USN Executive Director, TSNRP
	Bennetts, P., Shen, Q., Thimmesch, A., Clancy, R., Pierce, J. (2012) Effects of ubiquinol on reactive oxygen species and cellular injury in rats following hemorrhagic shock and fluid resuscitation. University of Kansas Medical Center, Student Research Forum, Kansas City, KS. 04 APR 2013	09 OCT 2012 John P. Maye, CRNA, PhD, CAPT, NC, USN Executive Director, TSNRP
	Shen, Q., Hiebert, J. Pierce, T., Clancy, R., & Pierce, J. Effects of Ubiquinol in Reducing Myocyte Hydrogen Peroxide and Mitochondrial Damage in Rats Following Hemorrhagic Shock &Fluid Resuscitation. University of Kansas Medical Center, Resident, Postdoc & Fellow Research Day, Kansas City, KS. 09 MAY 2013.	09 OCT 2012 John P. Maye, CRNA, PhD, CAPT, NC, USN Executive Director, TSNRP

	Shen, Q., Holloway, N., Thimmesch, A., Wood, J., Clancy, R., & Pierce, J. Ubiquinol Reduces Leukocyte-Endothelium Interactions in the Mesenteric Microcirculation Following Hemorrhagic Shock and Fluid Resuscitation. Experimental Biology Conference, San Diego, CA. April 26-30, 2014.	12 NOV 2013 Michael Schlicher, PhD, RN, LTC, AN Executive Director, TSNRP
Media Reports	TSNRP Newsletter	
Other	None	

Reportable Outcomes

Reportable Outcome	Detailed Description
Applied for Patent	None
Issued a Patent	None
Developed a cell line	None
Developed a tissue or serum repository	None
Developed a data registry	None

Recruitment and Retention Aspect	Number
Animals Projected in Grant Application	110
Animals Purchased	110
Model Development Animals	Not applicable
Research Animals	110
Animals With Complete Data	86
Animals with Incomplete Data	24

Final Budget Report

Table 4 summarizes how much monies were expensed in each category for the three-year grant period, no funds remain. Attached is an official final budget from the Kansas University Medical Center. There were sufficient funds for the expenses of this study. The debt acquired in the other operating expenses category was $5410.91 and $3,673.31 in the supplies category. This debt was covered by reallocating the remaining balance in the personnel category of $8,191.93 (other operating expenses: $4,518.62, supplies: $3,673.31). Following reallocation of funds to the other operating expenses category from the personnel category, a debt of $892.29 still exist and was covered by the reallocation of $780.73 from capital outlay $111.56 from the travel category. Since these amounts were less than 10% of the total amount of the awarded funds, an addendum was not necessary.

Table 4. The start, expensed, reallocated, and remaining balances in the personnel, travel, other operating expenses, supplies and capital outlay categories.

	Start Balance	Expensed	Reallocated	Remaining Balance
Personnel	$234,456.00	$226,264.07	$4,518.62 (to other operating expenses) $3,673.31 (to supplies)	$0.00
Travel	$2,000.00	$1888.44	$111.56 (to other operating expenses)	$0.00
Other Operating Expenses	$10,688.00	$16,098.91	$0.00	$0.00
Supplies	$17,686.00	$21,359.27	$0.00	$0.00
Capital Outlay	$34,985.00	$34,204.27	$780.73 (to other operating expenses)	$0.00
Total	$299,815.00	$299,815.00	$9,084.22	$0.00